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Evaluation of the Whole-Blood Alere Q NAT Point-of-Care RNA Assay for HIV-1 Viral Load Monitoring in a Primary Health Care Setting in Mozambique

Ilesh V. Jani,[a] Bindiya Meggi,[a] Adolfo Vubil,[a] Nádia E. Sitoe,[a] Nilesh Bhatt,[a] Ocean Tobaiwa,[b] Jorge I. Quevedo,[b] Osvaldo Loquiha,[c] Jonathan D. Lehe,[b] Lara Vojnov,[b] Trevor F. Peter[b]

Instituto Nacional da Saúde, Maputo, Mozambique[a]; Clinton Health Access Initiative, Maputo, Mozambique[b]; Department of Mathematics and Informatics, Universidade Eduardo Mondlane, Maputo, Mozambique[c]

Viral load testing is the WHO-recommended monitoring assay for patients on HIV antiretroviral therapy (ART). Point-of-care (POC) assays may help improve access to viral load testing in resource-limited settings. We compared the performance of the Alere Q NAT POC viral load technology (Alere Technologies, Jena, Germany), measuring total HIV RNA using finger prick capillary whole-blood samples collected in a periurban health center, with that of a laboratory-based plasma RNA test (Roche Cobas Ampliprep/Cobas TaqMan v2) conducted on matched venous blood samples. The whole-blood Alere Q NAT POC assay produced results with a bias of 0.8593 log copy/ml compared to the laboratory-based plasma assay. However, at above 10,000 copies/ml, the bias was 0.07 log copy/ml. Using the WHO-recommended threshold to determine ART failure of 1,000 copies/ml, the sensitivity and specificity of the whole-blood Alere Q NAT POC assay were 96.83% and 47.80%, respectively. A cutoff of 10,000 copies/ml of whole blood with the Alere Q NAT POC assay appears to be a better predictor of ART failure threshold (1,000 copies/ml of plasma), with a sensitivity of 84.0% and specificity of 90.3%. The precision of the whole-blood Alere Q NAT POC assay was comparable to that observed with the laboratory technology (5.4% versus 7.5%) between detectable paired samples. HIV POC viral load testing is feasible at the primary health care level. Further research on the value of whole-blood viral load to monitor antiretroviral therapy is warranted.

The World Health Organization strongly recommends the use of viral load testing as the primary tool for HIV antiretroviral treatment (ART) monitoring in its 2015 consolidated ART guidelines (1). The Joint United Nations Programme on HIV/AIDS (UNAIDS) has also highlighted the importance of viral load testing in its new 90-90-90 HIV targets and has launched a global initiative to increase access to viral load and other HIV diagnostic tests (2, 3). However, many resource-limited countries face challenges in implementing HIV viral load testing within their public health programs due to limited laboratory infrastructure, capacity, and skills. Emerging point-of-care (POC) technologies offer the opportunity to decentralize diagnostic testing and greatly expand access to quality health care (4).

Plasma-based RNA constitutes the conventional gold standard specimen type and biomarker for HIV viral load measurement. Currently, however, plasma-based testing requires venipuncture and processing of blood specimens using centrifugation to separate plasma, which require some laboratory infrastructure. The use of finger or heel prick capillary blood samples increases the ease of use of POC tests and enables both sample collection and viral load testing to be decentralized to locations that lack laboratories, required infrastructure, or skilled technicians.

The use of whole blood, although operationally advantageous, presents a technical problem, since both intracellular and extracellular RNA and DNA are present in the specimen and might be measured by the assay (5). Developers of POC viral load technologies are addressing this challenge either by building in an internal or external plasma separation step or by utilizing a more targeted biomarker. The Alere Q NAT POC assay (Alere Technologies, Jena, Germany) detects HIV-specific RNA and utilizes a small-volume whole-blood input cartridge (6, 7). This technology has recently been evaluated for the diagnosis of HIV in infants under 18 months of age in primary health care clinics, with promising results (8). We compared its technical performance and implications for clinical decision-making in primary health care with those of a laboratory-based plasma RNA viral load assay.

MATERIALS AND METHODS

Study participants. This was a blinded cross-sectional study where participants were tested using both POC- and laboratory-based viral load technologies. Adult HIV-positive patients at Polana Caniço Health Centre, Maputo, Mozambique, were invited to participate in the study. Only consenting patients were included in the study. In order to include patients with viral loads throughout all ranges (undetectable, detectable to 10,000 copies/ml, and greater than 10,000 copies/ml), patients were targeted for representation in those three ranges based on the following clinical information: on ART for longer than 6 months, on ART for between 4 weeks and 6 months, and on for ART less than 4 weeks, respectively. This clinic was selected based on its proximity to the HIV reference laboratory in Maputo and to facilitate study management and sample logis-

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Address correspondence to Ilesh V. Jani, ilesh.jani@gmail.com.

TABLE 1 Patient viral load sample demographics

Viral load (copies/ml)	No. (%) of patients by test[a]		
	Conventional ($n = 473$)	POC capillary ($n = 473$)	POC venous ($n = 473$)
Not detected[b]	210 (44.4)	153 (32.3)	55 (11.6)
<1,000	130 (27.5)	0 (0.0)	0 (0.0)
1,000–5,000	19 (4.0)	92 (19.5)	41 (8.7)
5,000–20,000	32 (6.8)	97 (20.5)	56 (11.8)
20,000–50,000	27 (5.7)	34 (7.2)	16 (3.4)
>50,000	54 (11.4)	57 (12.1)	32 (6.8)
Errors/not available	1 (0.2)	40 (8.5)	273 (57.7)

[a] Conventional, plasma laboratory-based testing; POC capillary, whole-blood finger prick Alere Q NAT; POC venous, whole-blood EDTA blood Alere Q NAT.
[b] Includes viral load counts below 20 for the conventional assay and viral loads equal to 0 for all POC assays.

TABLE 2 Means, medians, and ranges of detectable viral loads by test type[a]

Test	n	Viral load (copies/ml)		
		Mean	Median	Range
Conventional	190	76,244	7.853	21–1,580,511
POC capillary	190	125,297	18,250	1,050–2,890,000
POC venous	190	127,147	22,250	1,710–4,120,000

[a] Results are shown for only those samples that were tested on the conventional, POC capillary, and POC venous technologies.

tics. The clinic provided a range of HIV-related clinical services, including ART, and referred routine blood samples to the Instituto Nacional de Saúde HIV reference laboratory for viral load testing. Participants were enrolled and samples collected and tested between June 2012 and April 2013.

A unique randomly generated study identification number was assigned to each patient in order to link results from paired tests. Basic demographic data, including date of birth, gender, and date of ART initiation, were collected from all participants.

The study was approved by Mozambique's National Health Bioethics Committee, and all participants provided written informed consent before enrollment. Inclusion criteria included age over 18 years, documented HIV infection, and receipt of ART. Exclusion criteria included any serious medical conditions that could disrupt the accuracy of normal laboratory testing and its interpretation; however, no participants met this criterion. There was no exclusion on grounds of gender, socioeconomic status, race, or ethnicity.

POC and laboratory viral load testing. A capillary sample was collected from participants for immediate testing using a prototype of the Alere Q NAT POC device (Alere Technologies, Jena, Germany), which detects HIV-1/2 RNA (9). This point-of-care (POC) technology consists of a cartridge that collects 25 μl of whole blood and an instrument into which the cartridge is immediately inserted to run the assay in 60 min. No sample preparation or additional external steps are required. Sample preparation, reverse transcription, amplification, and detection are integrated within the cartridge. The technology specifically targets HIV RNA, while detection is based on competitive reported monitored amplification (CMA) technology. The technology is capable of detecting HIV-1 groups M, N, and O as well as HIV-2.

Two additional 5-ml venous blood samples in K_2 EDTA-evacuated tubes (Becton Dickinson, Franklin Lakes, NJ, USA) were collected from each patient. The blood sample was transported within 3 h to the Instituto Nacional de Saúde HIV reference laboratory, where it was separated by centrifugation to produce plasma within 6 h of collection. The plasma was frozen at −80°C before being tested using the Roche Cobas Ampliprep/Cobas TaqMan v2 (CAP/CTM) automated instrument (Roche Diagnostics, Branchburg, NJ, USA). Viral load testing was performed within 1 week of sample collection.

To evaluate the agreement between whole-blood POC viral load testing with the Alere Q NAT POC assay and laboratory-based plasma testing on the Roche CAP/CTM platform, we analyzed paired capillary whole blood and plasma derived from venous samples from patients, respectively.

Operators of both POC and laboratory viral load tests were formally trained on the respective technologies and were blinded to reciprocal results. The reference laboratory viral load instrumentation participated in and passed an external quality assurance program during the study and throughout the prior 12 months.

Statistical methods. Point-of-care and laboratory test results were $\log_{10}$ transformed. Undetectable and zero viral loads were assigned values of 1 copy/ml to enable log transformation and inclusion in the analysis. The results were compared using Pearson correlation, linear regression, and Bland-Altman assessment of agreement (average bias) (10) and limits of agreement (LOAs) (mean bias ± 1.96 standard deviations [SD]). The performance of the whole-blood Alere Q NAT POC assay for clinical ART patient management was assessed by determining its sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for identifying viral loads using several potential thresholds for treatment failure compared with laboratory-based plasma testing as the reference. Sensitivity and specificity were defined as the proportions of laboratory results above and below each threshold, respectively, that had concordant POC viral load results on the same patient. Precision of the laboratory and POC platforms was assessed using the coefficient of variation (CV) with a subset of 200 samples. Data were analyzed using SAS software v9.2 (SAS Inc., Cary, NC, USA), R v2.8.1, Microsoft Excel 2011 v14.1.0 (Microsoft Co., Redmond, WA, USA), and GraphPad Prism v6.0a (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

Patient demographics and clinical characteristics. A total of 443 study participants provided paired blood samples for both POC whole-blood and laboratory viral load analyses. Of these participants, 63.5% were female, 79.5% were between the ages of 20 and 50 years (range, 12 to 77 years).

The distribution of viral load results as measured by laboratory-based plasma testing ranged from undetectable to 1,772,416 copies/ml. Forty-four percent of patients had an undetectable viral load, 27% and 31% had detectable viral load results below 1,000 and 5,000 copies/ml, respectively, and 24% had viral loads above 5,000 copies/ml (Table 1).

Laboratory-based plasma testing showed a mean viral load of 33,638 copies/ml and a median viral load of 31 copies/ml. The whole-blood Alere Q NAT POC assay showed a mean viral load of 56,346 copies/ml and a median viral load of 4,200 copies/ml. Including only those samples with a detectable viral load, the laboratory-based plasma testing showed a mean viral load of 76,244 copies/ml and a median viral load of 7,853 copies/ml (Table 2). Including only those samples with a detectable viral load, the whole-blood Alere Q NAT POC assay showed a mean viral load of 125,297 copies/ml and a median viral load of 18,250 copies/ml. Ninety-four (47.7%) patients with undetectable viral loads by laboratory plasma-based testing had a detectable viral load by the whole-blood Alere Q NAT POC assay. Moreover, 50 (20.5%) patients with a detectable viral load by laboratory plasma-based testing had an undetectable viral load by the whole-blood Alere Q NAT POC assay, resulting in a sensitivity to detect nucleic acids of 79.5% (95% confidence interval [CI], 73.9 to 84.4%).

Agreement between capillary whole-blood Alere Q NAT POC and laboratory-based plasma viral load technologies. When all samples were analyzed, the correlation coefficient was

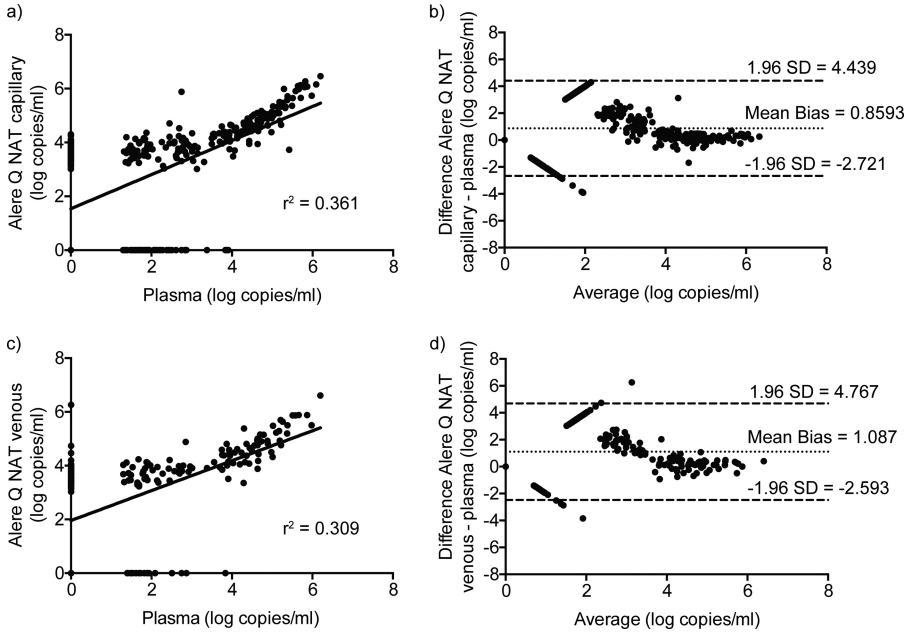

FIG 1 Analysis of agreement between the point-of-care whole-blood HIV viral load and laboratory-based plasma HIV viral load. Linear regressions and Bland-Altman comparisons of the paired Alere Q NAT capillary and conventional plasma samples (a and b) and paired Alere Q NAT venous and conventional plasma samples (c and d) for all patients on antiretroviral treatment are shown.

0.5961 ($r^2 = 0.361$), and the mean bias of the whole-blood Alere Q NAT POC assay was 0.8593 log copy/ml (95% LOA, −2.721 to 4.439 log copies/ml) (Fig. 1a and b). The whole-blood Alere Q NAT POC platform produced results in general agreement with laboratory-based plasma testing across the range of viral load levels, except for those below 10,000 copies/ml. When samples with laboratory-based plasma test results below 10,000 copies/ml were analyzed, the whole-blood Alere Q NAT POC test produced results with a mean bias of 1.056 log copies/ml (95% LOA, −2.758 to 4.869), while with samples with results above 10,000 plasma copies/ml, minimal mean bias was observed between the whole-blood Alere Q NAT POC assay and laboratory-based plasma testing (0.065 log copy/ml; 95% LOA, −0.582 to 0.712).

Misclassification analysis for capillary whole-blood Alere Q NAT POC and laboratory-based plasma viral load technologies. To evaluate the ability of the whole-blood Alere Q NAT POC assay to correctly identify virological failure in ART patients based on 2015 WHO consolidated ART guidelines, the sensitivity and specificity at the ART failure threshold of 1,000 copies/ml were calculated. The whole-blood Alere Q NAT POC assay had a sensitivity of 96.83% (95% CI, 92.07 to 99.13%) for identifying treatment failure using a threshold of 1,000 copies/ml compared with plasma laboratory testing; however, the specificity was 47.80% (95% CI, 42.19 to 53.44%) (Table 3). Furthermore, the Alere Q NAT POC assay had a positive predictive value of 42.36% and a negative predictive value of 97.44% using the ART failure threshold of 1,000 copies/ml (Table 3). At the threshold of 1,000 copies/ml, the mean viral load of the upward misclassified whole-blood Alere Q NAT POC samples was 11,190 copies/ml.

To identify a threshold with the whole-blood Alere Q NAT POC assay that classified virological failure in patients comparably to the laboratory-based plasma test, the ART failure threshold for the whole-blood Alere Q NAT POC assay was varied from 1,000 to 10,000 copies/ml and compared with patient categorizations with the laboratory-based plasma results using the WHO-recommended ART failure threshold of 1,000 copies/ml. The sensitivity of the whole-blood Alere Q NAT POC assay for detecting virological failure was 84.0% (95% CI, 76.4 to 89.9) at 10,000 copies/ml, while the specificity was 90.3% (95% CI, 86.4 to 93.3) (Table 3).

Agreement between venous Alere Q NAT whole-blood- and laboratory-based plasma viral testing technologies. To determine if capillary blood sampling had an influence on viral load

TABLE 3 Test performance for viral load counts for whole-blood capillary Alere Q NAT POC compared to plasma laboratory testing

POC capillary threshold (copies/ml)	No. by conventional laboratory testing (threshold, 1,000 copies/ml)				Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	True positive	False negative	True negative	False positive				
1,000	121	4	152	166	96.8	47.8	42.2	97.4
3,000	118	7	180	138	94.4	56.6	46.1	96.3
5,000	114	11	239	79	91.2	75.2	59.1	95.6
7,500	109	16	272	46	87.2	85.5	70.3	94.4
10,000	105	20	287	31	84.0	90.3	77.2	93.5

results with the whole-blood Alere Q NAT POC assay, results with venous whole-blood samples and the Alere Q NAT assay were compared to the plasma laboratory-based results with a subset of 207 ART patients. This comparison yielded a mean bias of 1.087 log copies/ml (95% LOA, −2.593 to 4.767) and an r^2 value of 0.309 (Fig. 1c and d). Using an ART failure threshold of 1,000 copies/ml, the sensitivity and specificity of the venous whole-blood Alere Q NAT assay were 98.39% and 37.93%, respectively, compared to the laboratory-based conventional plasma assay.

Precision analysis of whole-blood Alere Q NAT POC and laboratory-based plasma viral load technologies. To evaluate the precision of the capillary whole-blood Alere Q NAT POC assay, 96 duplicate finger prick capillary samples were collected in the clinic and analyzed immediately. Similarly, venous plasma samples from the same patients were analyzed in duplicate with the Roche CAP/CTM laboratory platform. The precision, as measured by the coefficient of variation, between duplicate Alere Q NAT whole-blood POC tests was 51.7% (95% CI, 41.0 to 62.5%), while the agreement of duplicate Roche CAP/CTM plasma-based tests was 17.9% (95% CI, 10.0 to 25.5%). The coefficient of variation was lower when sample pairs were excluded if the load in one duplicate in the pair was undetectable for both assays (for the Alere Q Nat whole-blood POC, CV = 5.4% [95% CI, 0.0 to 10.9%]; for the Roche CAP/CTM plasma-based platform, CV = 7.5% [95% CI: 2.0 to 13.3%]).

DISCUSSION

This study demonstrates that POC HIV viral load testing is operationally feasible at the primary health care level in resource-limited settings. The delivery of health care at the most peripheral levels of the health system plays an increasingly critical role in further scaling-up ART and forms a cornerstone of the UNAIDS 90-90-90 strategy. HIV viral load testing at the POC may also extend test access to settings where conventional laboratory assays are not easily accessible (11, 12). POC viral load tests may also facilitate the delivery of viral load-informed differentiated care for ART patients (13). Diagnostic testing at the POC improves test turnaround time, which may reduce the time to detection of treatment failure and the initiation of steps toward viral resuppression or therapy change (14–17). Prompt and appropriate clinical management of patients with suspected ART failure will be important for improving adherence, preserving the efficacy of treatment regimens, and preventing the spread of drug-resistant virus. Additional studies are needed to evaluate the potential benefit of POC viral load in these contexts (18, 19).

Plasma HIV RNA is the universal gold standard biomarker for detecting viral load quantification (20–22). The use of whole-blood samples, e.g., finger prick capillary whole blood or dried blood spots, can result in higher viral load measurements than the use of plasma due to the presence of cell-associated viral nucleic acid if not excluded during sample extraction or based on the test chemistry used (5). While the capillary whole-blood-based Alere Q NAT POC and plasma-based Roche CAP/CTM assays demonstrated good agreement in study participants with plasma viral load levels above 10,000 copies/ml, the Alere Q NAT assay produced higher test results in patients with plasma viral load below 10,000 copies/ml. The Alere Q NAT technology is an RNA amplification-based assay that measures both plasma and cell-associated RNA (total RNA). The contribution of the latter is likely the cause of higher whole-blood viral loads measured with this assay; however, this could also be due to unintended DNA amplification. As plasma-based HIV RNA and whole-blood total HIV RNA represent two different viral load biomarkers, direct comparisons are not straightforward.

The whole-blood Alere Q NAT POC technology failed to detect HIV RNA in 20.5% of patients who had detectable viral loads by the conventional laboratory-based technology. Though not investigated in this study, this may be due to the small sample volume used for this POC assay (25 μl of whole blood instead of the 500 μl of plasma used in most laboratory-based tests). Alternatively, the reduced detection by the Alere Q NAT POC technology could be due to lower analytical sensitivity of this assay. These technical issues may affect most of the POC viral load technologies that utilize whole blood collected from finger pricks.

A threshold of 10,000 copies/ml with the whole-blood POC assay had a sensitivity and a specificity of 94.4% and 85.1%, respectively, for detection of virological failure as defined by 1,000 copies/ml with the plasma-based laboratory viral load assay, suggesting that this higher-threshold whole-blood viral load would better correlate with the threshold for plasma viral load. With the population of patients in this study, the whole-blood POC assay at this threshold had positive and negative predictive values of 61.3% and 98.4%, respectively. The ability of this whole-blood POC assay to accurately classify virologically suppressed patients was inferior at lower hypothetical virological failure thresholds investigated in this study. Further investigations of this assay in different populations with different viral subtypes and in patients with different levels of viral suppression would be valuable.

Though current WHO guidelines recommend using viral load test data as a binary result above or below a specific virological failure threshold, determining ART success may be more appropriate using a trend analysis, as is commonly used to confirm suspected treatment failures in high-resource settings. Therefore, the use of viral failure trends may be a feasible approach, independent of which biomarker is used (22). In addition, it is important to identify biomarkers to monitor treatment adherence, an issue that already constitutes a significant public health concern in resource-limited settings. In this context, total RNA and/or cell-associated RNA may play a role by identifying new viral reservoirs resulting from low drug levels and the ensuing surge of viral replication. Additional research is required to evaluate the usefulness of whole-blood viral load and alternate biomarkers for the long-term monitoring of patients on ART.

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